

THE VIRULENCE OF TWO BIOINSECTICIDES (PROTECTO AND VIRUSET) AND THEIR EFFICACY ON SOME BIOLOGICAL ASPECTS OF *Spodoptera littoralis* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

Abdel-Salam, A. H.¹; A. M. Mabrouk² and N. M. Fayez²

¹ Economic Entomology Dept., Fac. of Agric., Mans. Univ., Mansoura

² Plant Protection Res. Institute, Agric. Res. Center, Dokki, Giza

ABSTRACT

Two commercial bioinsecticides; Protecto (*Bacillus thuringiensis* var. *kurstaki*) and Viruset (*Spodoptera littoralis* NPV) were bioassayed against the cotton leaf worm (CLW), *Spodoptera littoralis* (Boisd.) (2nd and 4th instar larvae). The obtained results reveal that Protecto was the most potent bioinsecticide compared with Viruset. Treatment of 2nd and 4th instar larvae with LC₅₀'s of Protecto and Viruset affected estimated biological aspects. The larval, pupal, and adult longevity durations were prolonged due to treatment. It was also observed that egg number and hatchability were affected by treatment.

INTRODUCTION

The cotton leafworm (CLW), *Spodoptera littoralis* is a polyphagous pest in Egypt. Without a hibernation period, cotton leafworm is active all year round, attacking cotton as well as more than 29 hosts from other crops and vegetables (Temerak, 2006). The rate of cotton leafworm infestation can reach up to 50,000 egg-masses/acres, causing severe damage to leaves, buds, flowers, and bolls (Temerak, 2006).

Development of an effective control method against the cotton leaf worm, *S. littoralis* is urgently needed since it does serious damage to many important agricultural crops in Egypt. There is a serious interest in the use of microbial insecticides for biological control of the cotton leafworm, as alternatives to chemical control, since they neither leave toxic chemical residues in the environment nor do they develop resistance in their insect hosts (Ahmed and El-Katatny, 2007). And hence, the public awareness and concern for environmental quality, has led to more focused attention on research aiming at developing biological agents. A promising strategy with good potential to control insect pests and, at the same time, to minimize the adverse effects of chemical insecticides is the use of microbial agents (Ahmed and El-Katatny, 2007).

These groups have unique modes of action and their properties may differ considerably from the conventional agents with whom growers are familiar (Asher, 1993 and Thompson *et al.*, 1999).

The virulence of two bioinsecticides; Protecto® (*Bacillus thuringiensis* var. *kurstaki*) and Viruset® (*Spodoptera littoralis* NPV [*Sp*lNPV]) on 2nd and 4th instar larvae of *S. littoralis* was evaluated. In addition, the efficacy of tested agents on some biological aspects of both larval instars was evaluated.

MATERIALS AND METHODS

Used insects:

A laboratory susceptible strain of the cotton leaf worm, *Spodoptera littoralis*, was reared for more than 10 generations. It was obtained from the Research Division of the cotton leaf worm, Plant Protection Research Institute. Insects were reared under controlled conditions in an incubator at $27 \pm 2^\circ\text{C}$, of $65 \pm 10\%$ R. H., and 8:16 L: D photoperiod at the Plant Protection Research Institute, Dokki-Giza, Egypt.

Chemical used:

Two commercial bioinsecticides; Protecto[®] (*Bacillus thuringiensis kurstaki*) and Viruset[®] (*Spodoptera littoralis* NPV) as wettable powders were evaluated for their toxicity on the CLW, *S. littoralis*. The tested bioagents were obtained from Plant Protection Research Institute Biopesticide Unit Production. Serial dilutions of Protecto and Viruset (9.4%) were prepared using 1g the wettable powder of each and dissolved in 100 ml of water.

Bioassay tests:

Two sets of five replicates each contain 10 newly molted 2nd and 4th instar larvae for each concentration of each tested product were used. Treatment of larvae was conducted by the leaf dipping technique. Fresh and clean castor leaves, *Ricinus communis* L., were immersed for 10 sec. in the prepared suspensions of the tested compounds. The treated leaves were then left to dry at room temperature before being offered to the newly molted 2nd and 4th instars of *S. littoralis* larvae. Larvae were left to feed on treated leaves for 48-h. They were then offered fresh clean leaves. The same numbers of larvae were used for check experiments in which larvae were offered fresh clean castor leaves dipped in water. Mortality was recorded daily and cumulative mortalities were recorded up till pupation.

Biological studies:

Larvae which survived treatment with LC₅₀ of the tested bioagents were observed for the following biological aspects: the duration of the rest of larval stage, pupation rate, duration of the pupal stage, adult emergence, reproductive potential of moths (fecundity per female and fertility per egg mass), and longevity of adult moths.

Statistical analysis:

Mortality rates were corrected according to Abbott's formula (Abbott, 1925) and plotted against concentrations as log/Probit regression lines. LC₂₅, LC₅₀, and LC₉₀ values as well as the slope of the lines were calculated (Finney, 1971) using "LdPLine[®]" software [http://embakr.tripod.com/ldpline/ldp_line.htm]. Means were tested for significance by the one way analysis of variance (ANOVA) using SPSS statistics 17.0 release 17.0.0 software.

RESULTS AND DISCUSSION

1-Virulence of tested compounds on 2nd and 4th instar larvae of *S. littoralis*:

Table (1) shows larval mortality rates due to treatment of the 2nd and 4th larval instars of *S. littoralis* with different concentrations of the tested compounds. LC₂₅, LC₅₀, and LC₉₀ values for both instars were determined. Protecto exhibited higher effectiveness than Viruset for both instar larvae.

Table (1). Susceptibility of the 2nd and 4th larval instars of the cotton leaf worm, *S. littoralis*, to the tested compounds

Tested compounds	Larval instar	LC ₂₅ (gm/ml)	LC ₅₀ (gm/ml)	LC ₉₀ (gm/ml)	Slope ± S.E.
Protecto	2 nd	8.7×10^{-8}	1.7×10^{-5}	0.418	0.29 ± 0.0297
	4 th	1.4×10^{-8}	2×10^{-5}	1.98	0.21 ± 0.0225
Viruset	2 nd	4.8×10^{-9}	1.1×10^{-5}	2.34	0.20 ± 0.0252
	4 th	4.7×10^{-7}	1×10^{-4}	3.134	0.29 ± 0.0315

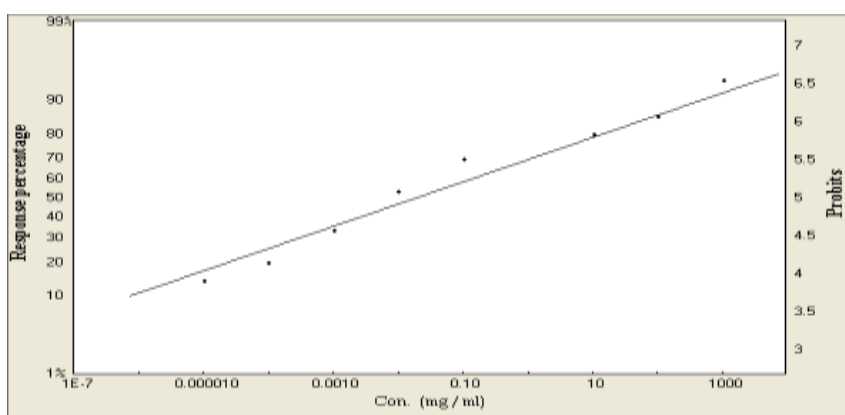


Fig. (1): Toxicity of Protecto[®] on 2nd instar larvae of *S. littoralis*.

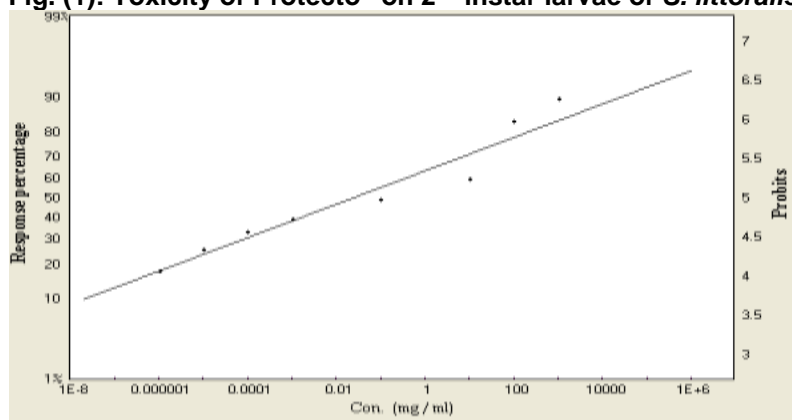


Fig. (2): Toxicity of Protecto[®] on 4th instar larvae of *S. littoralis*.

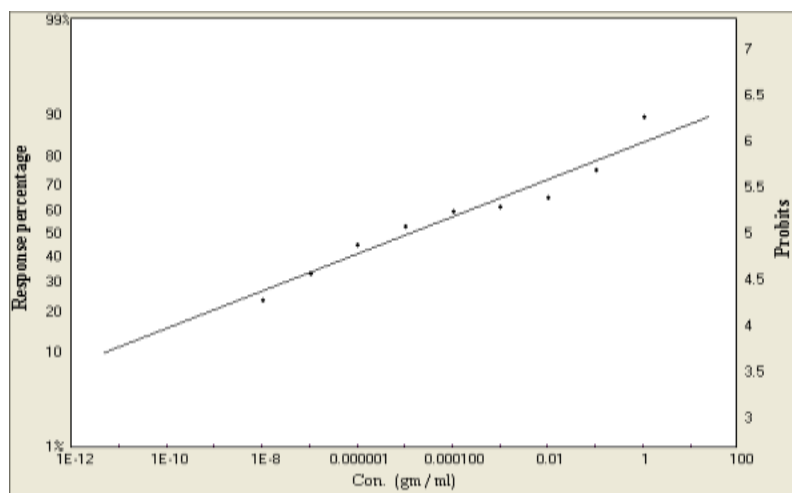


Fig. (3): Toxicity of Viruset® on 2nd instar larvae of *S. littoralis*.

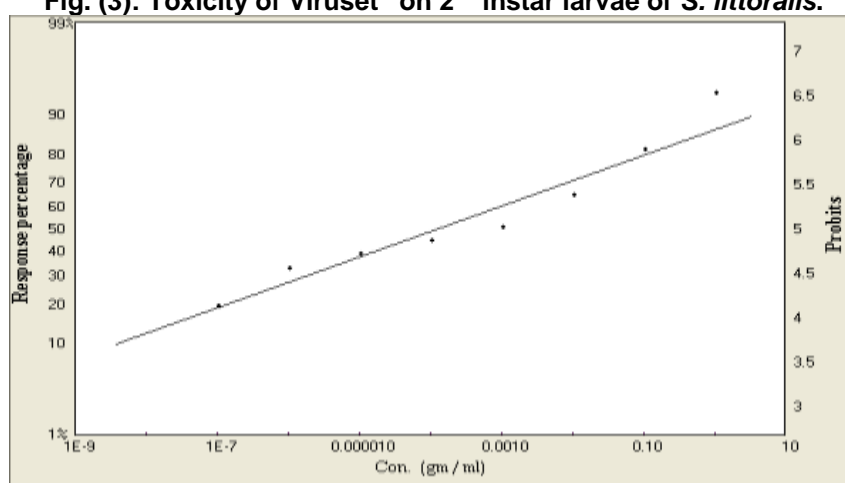


Fig. (4): Toxicity of Viruset® on 4th instar larvae of *S. littoralis*.

The tested bioagents did not result in instant mortalities 48 hrs. post treatment. However, mortality rates were increased at the termination of the larval stage. Second larval instar showed higher susceptibility to all the tested compounds than the 4th larval instar. This might be due to differences in sizes and defense mechanisms between instars. It is well documented that older instars of the cotton leaf worm are able to tolerate the toxic effect of these bioagents. Similar observations were reported by Hanafy *et al.* (2005); Abdel-Aziz (2007); and Abd El-Kareem (2007).

2- Effect of tested compounds on some biological aspects:

Treatment of the 2nd and 4th instar larvae with Protecto and Viruset prolonged the mean duration of the larval stage (Table 2).

Table (2). Effect of larval treatment with LC₅₀ of Protecto and Viruset on larval duration, pupation rate, and pupal duration of *S. littoralis*

Compounds	Mean larval duration (days) ± S. E.		%Pupation		Mean pupal duration (days) ± S. E.	
	2 nd	4 th	2 nd	4 th	2 nd	4 th
Protecto	14.5±0.1	12.0±1.7**	67.5	47.0	11.3±1.5***	9.3±1.3***
Viruset	16.0±0.5	11.6±0.6	52.5	56.6	11.3±1.5***	10.0±1.7***
Check	15.0±0.2	10.3±1.1	100	100	13.6±0.5	14.0±1.7

*: Significant at P> 0.05 **: highly significant at P> 0.01 ***: Very highly significant at P> 0.001

The mean pupal duration was reduced as a result of treatment with the tested compounds. In addition, there was a decrease in adult emergence and mean adult longevity (Table 3). Mohamed (2006) reported a similar delay of ecdysis in larvae treated with NPV. Atwa *et al.* (1984) showed that latent effects of *Bt* on treated insects manifested as decrease of pupation and adult emergence. Abd El-Halim (1993); Abd El-Latif (2001); Dutton *et al.* (2003); Gamil (2004) and Mohamed (2006) also found that the development time of larvae and pupae were extended as well as adult emergence after treatment with bacterial or viral agents.

Table (3). Effect of larval treatment with LC₅₀ of Protecto and Viruset on adult emergence and longevity of *S. littoralis*

Tested compounds	% Adult emergence		Mean adult longevity (days) ± S. E.			
	2 nd	4 th	2 nd		4 th	
			♂	♀	♂	♀
Protecto	94.40	94.20	8.3±1.15***	9.3±0.57***	14.3±1.2**	12.0±1.7***
Viruset	100.00	94.10	9.0±1.0***	10.3±0.57**	14.0±1.1**	13.0±1.5***
Control	100.00	100.00	13.6±1.15	14.6±0.58	17.0±1.0	15.3±0.57

*: Significant at P> 0.05 **: highly significant at P> 0.01 ***: Very highly significant at P> 0.001.

Reproductive potential of *S. littoralis* moths treated as 2nd or 4th instar larvae with LC₅₀ of the tested bioagents was reduced (Table 4). It is likely that the used compounds interfered with egg formation or development and consequently, led to reduction in the number of laid eggs. Results obtained could be explained by those reported by Santiago-Alvarez and Osuna (1988) and Aldebis *et al.* (1993). They found that males of *S. littoralis* infected with NPV and allowed to mate with untreated females produced normal number of eggs but showed a significant reduction in egg hatchability. In addition, the observed reduction in the percentage of egg hatch may be attributed to impairment of either eggs and/or sperms as a result of treatment. Furthermore, it may be due to inability of the sperms to be transferred to the females during copulation, as suggested by Ismail (1980) and Aldebis *et al.* (1993). Many researchers reported a low reproductive capacity in the cotton

leafworm moths treated with bioagents (Hassan, 2004; Mohamed *et al.*, 2005; Hatem, 2006; Abdel-Aziz, 2007; and Abd El-Kareem *et al.*, 2010).

Table (4): Effect of larval treatment with LC₅₀ of Protecto and Viruset on fecundity and fertility of *S. littoralis*

Tested compounds	Mean no. of eggs/female \pm S.E.		Mean no. hatched eggs/female \pm S.E.	
	2 nd	4 th	2 nd	4 th
Protecto	422 \pm 12.5	698 \pm 14.2	282 \pm 4.7	646 \pm 15.3
Viruset	847 \pm 16.8	671 \pm 18.4	528 \pm 8.5	622 \pm 4.04
Control	2135 \pm 60.6	1875 \pm 15.1	2103 \pm 4.04	1857 \pm 12.11

***: Very highly significant at $P > 0.001$.

REFERENCES

- Abbott, W.S. (1925). A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265- 277.
- Abd El-Kareem, S. M. I. (2007). Biological and histopathological studies on the effect of certain entomopathogenic microorganisms on the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) M. Sc.Thesis, Fac. Sci., Ain Shams Univ. Pp. 187.
- Abdel-Aziz, M. M. M. (2007). Controlling of the cotton leafworm, *Spodoptera littoralis* (Boisd.), by using environmentally safe (nontraditional) methods. M. Sc. Thesis, Institute of Environmental Studies and Research, Ain Shams University. Pp. 111.
- Abd El-Halim, S. M. (1993). Bioactivity of Dipel 2X, Commercial preparation of *Bacillus thuringiensis* (Berliner) against the cotton leaf worm, *Spodoptera littoralis* (Bosid). Egypt. J. Agric. Res., 7 (1): 307-315.
- Abd El-Latif, E. M. A. (2001). Integrated pest Management for cotton in Dakahlia governorate. Ph. D. Thesis, Fac. Agric. Mansoura Univ. Pp. 180.
- Aldebis, H. K.; Osama, E.V. and Santiago Alvarez, C. (1993). Development of the male reproductive system in *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) treated as larvae with nuclear polyhedrosis virus (Baculoviridae). Boletin de Sanidad Vegetal, Plagas. 19 (1): 3 – 9.
- Ahmed, A. M. and El-Katatny, M. H. (2007). Entomopathogenic fungi as biopesticides against the Egyptian cotton leafworm, *Spodoptera littoralis*: between biocontrol-promise and immune-limitation. Journal of Egyptian society of toxicology, 37: 39-51.
- Asher, K. R. S. (1993). Nonconventional insecticidal effects of pesticides available from the Neem tree, *Azadirachta indica*. Archives of insect biochemistry and physiology, 22(3-4): 433-449.
- Atwa, W. A.; Abdel-Rahman, M. A. and El-Sharkawy, A. Z. (1984). Effect of *Bacillus thuringiensis* Berliner on the biology of Eri-silkworm *Philosamia ricini* (Boids.) (Lepidoptera: Salurimlidae). 5th Annual conference of the African Association of Insect Scientists. pp. 13-21.
- Dutton, A.; Klein, H.; Romeis, J. and Bigler, F. (2003). Pre-mediated effects of *Bacillus thuringiensis* spray on the predator *Chrysoperla carnea* in maize. Biol. Control. 26(2): 209-215.

- Finney, D. J. (1971). Probit Analysis, A statistical treatment of the sigmoid response curve 7th Ed., Cambridge Univ. Press, Cambridge, England
- Forgash, A. J. (1984). History, evolution and consequences of insecticide resistance. *Pestic. Biochem. Physiol.*; 22:178–186.
- Gamil, W. E. (2004). Production of some bioformulations and study of their efficiency on some physiological traits in some insect species M. Sc. Thesis, Fac. Agric., Ain Shams Univ. Pp. 124.
- Georghiou, G. P. (1986). The magnitude of the resistance problem. p. 14–43. In: "Pesticide Resistance: Strategies and Tactics for Management". National Academy Press, Washington, D.C.
- Hanafy, H. G. M.; Sakr, H. E. A. and El-Sayed, W. (2005). Toxicological and biochemical effects of bioagents products on the cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Arab Univ. J. Agric. Sci.*; Ain Shams Univ., Cairo, 13(3): 939-950.
- Hassan, M. M. (2004). New approach for controlling the cotton leaf worm, *Spodoptera littoralis*. M.Sc. Thesis, Fac. Science, Mansoura Univ., Egypt.
- Hatem, A. E. (2006). Comparacion de los efectos de insecticidas selectivos sobre el desarrollo y reproduccion de *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Thesis Dextoral Universided de Cardoba.240.
- Ismail, I. E. (1980). Physiological studies on the effect of juvenile hormone analogues upon the cotton leaf worm, *Spodoptera littoralis* (Boisd.). Ph. D. Thesis, Cairo Univ.
- Mabrouk, A. M. and El-Abbas, F.A. (2002). Effectiveness of nuclear polyhedrosis virus against the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Arab Univ. J. Agric. Sci.*, Fac. Agric., Ain Shams Univ., 10(1): 391-403.
- Mohamed, E.H. (2006). The use of three entomopathogens for controlling the cotton leaf worm *Spodoptera littoralis* (Boisd.) in the Egyptian clover fields as affective contribution to IPM programs. M. Sc. Thesis, Fac. Agric., Cairo Univ.
- Rao, N. V.; Reddy, A. S.; and Reddy, P. S. (1990). Relative efficacy of some new insecticides on insect pests of cotton. *Indian J. Plant Prot.* 18: 53–58.
- Santiago-Alvarez, C. and Osuna, E.V. (1988). Reduction of reproductive capacity of *Spodoptera littoralis* males by a nuclear polyhedrosis virus (NPV). *J. Invert. Pathol.* 52: (1): 142- 146.
- Sawicki, R. M. (1986). Resistance to synthetic pyrethroids can be countered successfully. *Agribusiness Worldwide* 8: 22–25.
- Temerak, S. A. (2006). Historical records of application of bio-control agents and IPM to combat cotton leafworm and cotton bollworms with special reference to the hazard of conventional insecticides from 1900-2006. *World j. Agric. Sci.*, 2(3): 227-232.

Thompson, G. D.; Hutchins, S. H.; and Sparks, T. C. (1999). Development of spinosad and attributes of a new class of insect control products. Univ. of Minnesota. Available at: <http://www.ipmworld.umn.edu/chapters/hutchins2.htm>

فاعلية مركبان حيويان (بروتكتو وفيروست) وتأثيرهما على بعض المظاهر البيولوجية لدودة ورق القطن الكبرى
عادل حسن عبد السلام¹، آمال محمد مبروك² و نيفين محمد فايز²
1 قسم الحشرات الاقتصادية، كلية الزراعة، جامعة المنصورة، المنصورة
2 معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقى، جيزة

تم تقييم تأثير مركبان حيويان هما بروتكتو (*Bacillus thuringiensis* var. *kurstaki*) وفيروست (*Spodoptera littoralis*/NPV) على يرقات العمرين الثانى والرابع لدودة ورق القطن. من النتائج التى تم الحصول عليها وُجد أن مركب بروتكتو كان أكثر المركبات فاعلية مقارنة بمركب فيروست. أشارت الدراسة إلى أن تأثير المعاملة بكل من مركبى بروتكتو وفيروست العمرين الثانى والرابع بالتركيز القاتل للنصف (LC₅₀) أطال فترات العمر اليرقى، وعمر العذارى، وعمر الحشرات الكاملة نتيجة المعاملة. كما وُجد أيضاً أن عدد البيض الموضوع ونسبة الفقس قد تأثرتا بشكل واضح نتيجة المعاملة.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
مركز البحوث الزراعيه

أ.د / سمير صالح عوض الله
أ.د / سندس عبد التواب محمد